Docket No.48340 (49663)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT:	H. Belmont, et al.		
SERIAL NO.:	10/024,648	EXAMINER:	A. Wehbe
FILED:	12/19/2001	GROUP:	1632
FOR:	TRANSGENIC ANIMALS COMPRISING A HUMANIZED IMMUNE SYSTEM		

DECLARATION OF HEATHER BELMONT UNDER 37 C.F.R. §1.132

Dear Madam:

1. I, **HEATHER BELMONT**, declare and say that I am a resident of the United States. My residence address is 19810 NE 22 Ave., North Miami Beach, FL 33180.

2. I currently hold the position of Scientist at Altor BioScience Corporation of Miramar, Florida. I have special expertise and knowledge in the field of transgenic mice including how to make and use such animals. My Curriculum Vita is attached as **Appendix B**.

3. I am a co-inventor of claims 1-8, 30, 31, 38-47, 112 and 113 as set forth in the above-captioned application ("subject application"). I personally performed, directed, and/or assisted in research leading to the claimed invention.

4. I read the Office Action dated June 16, 2004 in the application. I understand from the Office Action that the USPTO rejected claims of the application on grounds that the subject application does not show how to make and use the claimed

invention. I respectfully disagree with the USPTO. Indeed, my specification shows how to make and use the invention claimed. My reasons follow.

5. For instance, I am familiar with unpublished research that was performed by me and my co-inventors or under my direction. That work was conducted along lines of my patent specification and it showed, among other things, that one could produce transgenic mice that include unrearranged human TCRα loci that include multiple V, J, and C gene segments.

6. The unpublished research of which I am familiar further shows, among other things, that the transgenic mice can include human TCRα loci with human regulatory elements (ie., promoters, enhancers, splice sites, poly A sites and others). The research further shows that the elements are functional in the mice ie., they can direct rearrangement and expression of human TCRα transcripts in thymocytes of HuTCRα transgenic mice.

7. In accord with the subject application, one working in the field can readily make and use such transgenic mice. For instance, Figure 4 and its supporting disclosure provide a detailed overview of the main procedural steps used in the construction of certain T-cell receptor alpha constructs. Methods for incorporating transgenes into appropriate germlines are also provided. See pgs. 20-21, bridging paragraph. Other particular transgenic methods are disclosed as well. See pg. 21, lines 23-36. Strategies for inactivating germlines via "knockout" technologies are also provided. At pg. 21, last line to pg. 24, line 27. Preferred primers, vectors, and other suitable nucleic acid reagents have been provided (see pg. 24, line 29 to pg. 26, line 15) as well as illustrative mouse strains for use with the invention. At pg. 26, lines 17-24. Various TCR-based materials have also been disclosed. See pg. 24, line 26 to pg. 30, line 20. Production and use of resulting mouse strains have also been provided. See pg. 30, line 22 to pg. 32, line 22.

8. The subject application further provides ten working examples that show how to make and use (1) mice with inactivated alpha and beta TCR chains, (2) vectors that express human TCR, and (3) mice that express human TCR genes. See Examples 1-7, 10. Use of such mice is provided in Example 8, for instance. The specification also provides how to make and use HLA expressing mice in Example 9.

9. Consistent with the detailed information provided by the subject application, me and my co-inventors performed and/or directed the following unpublished research.

10. **Production of human TCRα Yeast Artificial Chromosome (YAC) Vector**

Me and my co-inventors constructed human TCRα and TCRβ transgenes using information obtained from the NCBI and National Human Genome Research Institute databases. Specifically, we accessed databases that disclosed the sequence of human TCR gene loci. We identified human YACs containing genomic human T gene fragments from several commercially available YAC libraries. For the HuTCRα gene a 700 Kb YAC that contained the majority of the HuTCR alpha genomic fragment spanning from TRAV13-2 though the 3' UTR was used as the unrearranged HuTCRα transgene. For the HuTCRβ transgene a 325 Kb YAC that contained the majority of the HuTCR beta genomic fragment spanning from TRBV12-3 though the 3' UTR was used as the unrearranged HuTCRβ transgene. Both YACs were isolated from the endogenous yeast chromosome background via pulse-field gel electrophoresis. Guidance on the construction and use of appropriate YAC vectors can be found throughout the subject application. See eg., pg. 25, lines 16-18; and Examples 3-6.

11. **Injection of TCRα and HuTCRβ YAC Vector Into Mouse Embryo And Production of Transgenic Mice**

Me and my co-inventors injected, or had injected under our supervision, one cell stage embryos following techniques generally outlined in our specification. See eg., See

pgs. 20-21, bridging paragraph, of the specification. See also Examples 4-6 of the specification (in particular, citing Hogan, et al., "Manipulating the Mouse Embryo: A Laboratory Manual", Cold Spring Harbor Laboratory, and Montolui, L. (1996) in Methods in Molecular Biology, Vol.54: YAC Protocols. Eds. D. Markie. Humana Press Inc., Tolowa, NJ.) Resulting offspring were tested for integration of the either of the HuTCRα YAC or the HuTCRβ_YAC transgenes by PCR analysis of tail biopsies. One heterozygous founder mouse injected with the HuTCRβ YAC had a complete integration event spanning the entire HuTCRβ YAC transgene. This mouse line can be bred to homozygosity. Another heterozygous founder mouse injected with the HuTCRα YAC had an integration event that spanned 10 TRAV segments, all 61 TRAJ segments, and the TRAC gene segment of the HuTCRα gene (approximately 400 Kb). This founder was bred to homozygosity and the resulting offspring were tested for potential functional human TCRα gene rearrangements.

12. **Transgenic Mice Produce Human TCRα Transcript**

RNA was isolated from thymus or spleen of adult HuTCRα transgenic mice or Bl/6 control mice and used as templates for cDNA synthesis in accord with disclosure provided throughout the subject application. For instance, see pg. 53, line 16 to 28. cDNA was subjected to RACE (5'-rapid amplification of cDNA ends) reactions followed by PCR using various primers that extend through the huTRAV and the huTRAC gene segments of the HuTCRα YAC transgene. Resulting amplimer products indicate that the HuTCRα YAC is expressed and creates rearranged human TCRα transcripts. More specifically, when PCR reactions were performed on RACE cDNAs derived from C57Bl/6 (Bl/6 control) or HuTCRα transgenic mouse thymus tissues using primer pairs huTCRAV36/DV7 and huTCRAC, huTCRAV38-2 and huTCRAC, or huTCRA forward and reverse, we observed amplimers only in the RACE amplified cDNAs derived from HuTCRα homozygous thymus tissue (**Appendix A**).

This data is consistent with the human TCRα transcripts being present only in HuTCRα transgenic mice and indicates that multiple rearrangements occurred since several different human TCR gene primer pairs created amplimer products. Primer pair huTCRAV38-2 and huTCRAC was also able to amplify products from HuTCRα

transgenic adult spleen RACE cDNAs (**Appendix A**). This suggests that the process of thymocyte maturation, which includes positive and negative selection, occurred in the thymi of HuTCRα transgenic mice. We have sequenced several of the amplimer products from the PCR reactions and have confirmed that at least four human TCRα V gene segments (huTCRAV26-2, huTCRAV34, huTCRAV36/DV7 and huTCRAV38-2) align with various TCRAJ segments as well as the TCRAC segment to create complete human TCRα transcripts.

13. **Appendix A** are photographs showing amplified DNA separated by gel electrophoresis and visualized with Ethidium Bromide. Each lane is explained in more detail as follows:

From Photograph (A) –

Lanes 1, 6, and 10 - show that RACE cDNA derived from non-transgenic Bl/6 mouse thymus RNA does not create amplimers in the presence of the huTCRA primer sets.

Lanes 2 - shows that RACE cDNA derived from HuTCRα transgenic mouse #$10^{+/+}$ thymus RNA creates amplimers of the appropriate size in the presence of the huTRAV36/DV7 and huTRAC primer set.

Lanes 3 - shows that RACE cDNA derived from HuTCRα transgenic mouse #$14^{+/+}$ thymus RNA creates amplimers of the appropriate size in the presence of the huTRAV36/DV7 and huTRAC primer set.

Lane 7 - shows that RACE cDNA derived from HuTCRα transgenic mouse #$10^{+/+}$ thymus RNA creates amplimers of the appropriate size in the presence of the huTRAV38-2 and huTRAC primer set.

Lane 8 - shows that RACE cDNA derived from HuTCRα transgenic mouse #$14^{+/+}$ thymus RNA creates amplimers of the appropriate size in the presence of the huTRAV38-2 and huTRAC primer set.

Lane 11 - shows that RACE cDNA derived from HuTCRα transgenic mouse #$10^{+/+}$ thymus RNA creates amplimers of the appropriate

size in the presence of the huTRAC forward and reverse primer set.

Lane 12 - shows that RACE cDNA derived from HuTCRα transgenic mouse #14$^{+/+}$ thymus RNA creates amplimers of the appropriate size in the presence of the huTRAC forward and reverse primer set.

Lane 15, 16, and 17 - show that the RACE cDNAs generated from the control Bl/6 and HuTCRα transgenic mouse thymus RNA are intact and can be used as templates for PCR since the positive control primer pair for mouse glyceraldehydes 3-phosphate dehydrogenase (GAPDH) creates appropriately sized amplimers.

Lane 4, 9, 13, and 18 - are negative control samples that show that the PCR master mixes are not contaminated and do not create amplimers without the addition of RACE cDNA.

Lane 5 and 14 – show 100 bp markers to estimate amplimer size.

From photograph (B) -

Lanes 1 - shows that RACE cDNA derived from non-transgenic Bl/6 mouse spleen RNA does not create amplimers in the presence of the huTRAV38-2 and huTRAC primer set.

Lane 2 - shows that RACE cDNA derived from HuTCRα transgenic mouse #10$^{+/+}$ thymus RNA creates amplimers of the appropriate size in the presence of the huTRAV38-2 and huTRAC primer set.

Lane 3 - shows that RACE cDNA derived from HuTCRα transgenic mouse #14$^{+/+}$ thymus RNA creates amplimers of the appropriate size in the presence of the huTRAV38-2 and huTRAC primer aet.

Lane 4 and 9 - are negative control samples that show that the master mixes are not contaminated and do not create amplimers without the addition of RACE cDNA

Lane 6, 7 and 8 - show that the RACE cDNAs generated from the control Bl/6 and HuTCRα transgenic mouse RNA are intact and can be used as templates for PCR since the positive control primer set for

mouse glyceraldehydes 3-phosphate dehydrogenase (GAPDH) creates appropriately sized amplimers.

Lane 5– shows the 100 bp markers to estimate amplimer size.

14. In sum, one reading the subject application would understand that it shows how to make and use transgenic mice that includes human alpha and beta chains. More specifically, the mice would be understood to have functional human transgenes with unrearranged loci under the control of human regulatory sequences. See also **Appendix A** and the subject application particularly under the Summary of the Invention section

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

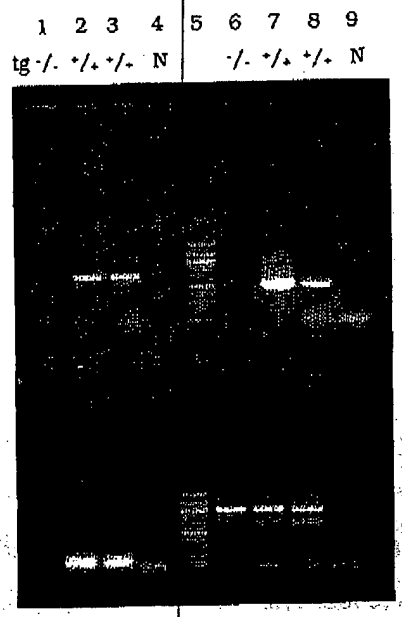
2-18-05
Date

Heather J Belmont
Heather J. Belmont

Doc. 473357

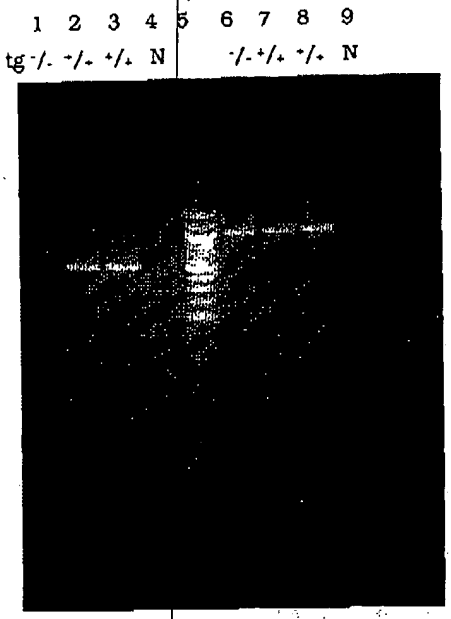
Appendix A

(A) Adult Thymus



[redacted]

(B) Adult Spleen



Appendix A: **(A)** PCR of RACE cDNA products derived from mouse thymus RNA using primers specific for huTCRAV36/DV7 and huTCRAC (Lanes 1-4), huTCRAV38-2 and huTCRAC (Lanes 6-9), human TCRA constant region with primers TCRA forward and TCRA reverse (Lanes 10-13) and positive control primers for mouse GAPDH (Lanes 15-19). **(B)** PCR of RACE cDNA products derived from mouse spleen RNA using primers specific for huTCRAV38-2 and huTCRAC (Lanes 1-4) and positive control primers for mouse GAPDH (Lanes 6-10). tg = indicates presence or absence of the HuTCRα transgene, N = No RACE cDNA control PCR reaction

Appendix B

HEATHER J BELMONT

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EXPERIENCE

2004- present **Scientist,** Research and Development, Altor BioScience Corporation, Miramar, FL.

- Develop and conduct *in vivo* efficacy and toxicity studies for anti-tumor TCRs
- Develop research strategy for TCR cancer group including milestones
- Establish protocols for soluble T-cell receptor *in vitro* diagnostic reagents
- Create and characterize transgenic mice expressing genomic human T-cell receptor (TCR) genes
- Institutional Animal Care and Use Committee Chairperson
- Write and edit manuscripts and patent applications

2003- 2004 **Associate Scientist,** Research and Development, Altor BioScience Corporation, Miramar, FL.

- Create and characterize T-cell based immunotherapeutics for prostate cancer-specific antigens
- Supervise technician responsible for characterization of several new TCR-based molecules
- Established Altor's Institutional Animal Care and Use Committee including all regulatory submissions to OLAW and NIH

2001-2003 **Associate Scientist,** Research and Development, Sunol Molecular Corporation, Miramar, FL.

- Conduct independent research to create transgenic animals that express human T-cell receptor genes
- Write and edit patent application
- Establish animal models to study the efficacy of immunotherapeutic agents on primary tumor growth and metastasis

- Design and supervise construction of Sunol's animal facility

1996-2001 **Ph.D. Candidate,** University of Miami, Miami, FL.

- Develop and characterize multiple transgenic mouse models to investigate growth factor control of cardiovascular and nervous system development
- Characterize transgenic mice with aberrant expression of Steel Factor
- Investigate the role of growth factors in peripheral nerve tumor formation
- Prepare and present lectures on embryonic development and neurophysiology for undergraduate and medical students
- Train new graduate and new graduate students

1992- 1994 **Research Assistant,** Ithaca College, Ithaca, NY.

- Determine factors that motivate spending behaviors in human subjects
- Work led to first professional publication and personal interviews with the Washington Post, Associated Press, and Men's Health magazine

EDUCATION

2001 Ph.D., University of Miami School of Medicine, Miami, FL.
Interdisciplinary Neuroscience Program
Mentor, Dr. Brian Masters

1994 B. A., Ithaca College, Ithaca, NY
Psychology and Neuroscience
Mentors, Drs. Michael McCall and Jack Peck
Magna cum Laude

HONORS AND AWARDS

- Pre-Doctoral Fellowship, American Heart Association, "Understanding Cardiovascular Congenital Malformations: The role of PDGFA in Cephalic Neural Crest Cell Development", 1997-2000
- University of Miami Graduate Student of the Year Award, Miami, FL, 2000
- Best Oral Presentation, Eastern Student Research Forum, Miami, FL, 2000
- Student Award for Best Poster Presentation, Southeast Society for Developmental Biology, Atlanta, GA, 2000
- ALPHA EPSILON LAMDA, National Graduate Student Honor Society, inducted 1996
- PHI KAPPA PHI, National Honor Society, inducted 1993

- PSI CHI, National Psychology Honor Society, inducted 1993
- Dana Student Internship for Research, Ithaca College, Ithaca, NY, 1992
- Leonard B. Job Scholarship, Ithaca College, Ithaca, NY, 1992-94
- Lyons Falls Scholarship, 1990-94

PUBLICATIONS

Price-Schiavi SA, Belmont HJ, Zhu X, Card KF, and Wong HC. In vivo efficacy of a soluble single-chain T-cell receptor IL-2 fusion protein. Manuscript under revision.

Mosquera LA, Card KF, Price-Schiavi SA, Belmont HJ, Liu B, Builes J, Zhu X, Chavaillaz PC, Lee H-I, Jiao J-J, Francis JL, Amirkhosravi A, Wong RL, and Wong HC. *In vitro* and *in vivo* Characterization of a Novel Antibody-Like Single-Chain T-cell Receptor Human IgG1 Fusion Protein . Journal of Immunology, (2005) in press.

Card KF, Price-Schiavi SA, Liu B, Thomson E, Nieves E, Belmont HJ, Builes J, Jiao JA, Hernandez J, Weidanz J, Sherman L, Francis JL, Amirkhosravi A, Wong HC. A soluble single-chain T-cell receptor IL-2 fusion protein retains MHC-restricted peptide specificity and IL-2 bioactivity. Cancer Immunology Immunotherapy. 2004 April 53(4): 345-57.

Belmont, HJ. Growth Factor Participation in Neural Crest Cell Patterning. (2001) Ph.D. Dissertation, University of Miami, Coral Gables, FL.

McCall, M. and Belmont, HJ. Credit Card Insignia and Restaurant Tipping: Evidence for an Associative Link. Journal of Applied Psychology. (1996) 81(5): 609-613.

PRESENTATIONS

Card KF, Belmont HJ, Zhu X, , Liu B, and Wong HC. T-cell receptor single-chain fusion proteins as immunomodulatory cancer therapies. (2005) Keystone Symposium, Keystone, CO.

Zhu X, Belmont HJ, Price-Schiavi SA, Liu B, Lee H-I, and Wong HC. A tetrameric soluble single chain T-cell receptor fusion protein can bind tumor cells in a MHC-restricted and peptide-specific manner. (2005) American Association for Cancer Research, Poster Presentation, Anaheim, CA.

Belmont HJ, Mosquera, LA, Card KF, Price-Schiavi SA, Builes J, and Wong, HC

A novel soluble single-chain T-cell receptor IgG1 fusion protein retains MHC restricted peptide specific antigen binding and exhibits anti-tumor effects *in vivo*. (2004) American Association for Cancer Research, Poster Presentation, Orlando, FL.

Price-Schiavi, SA, Belmont, HJ, Card, KF, Builes, J, and Wong, HC. Anti-human tumor activity of a soluble, single-chain T-cell receptor IL-2 fusion protein in mouse xenograft models. (2004) American Association for Cancer Research, Poster Presentation, Orlando, FL.

Belmont, HJ. Growth factor participation in neural crest cell patterning. (2001) Sunol Molecular, Oral Presentation, Miramar, FL.

Belmont, HJ. The role of Platelet-Derived Growth Factor A-chain in patterning of cephalic neural crest. (2000) Eastern Student Research Forum, Oral Presentation, Miami, FL.

Belmont, HJ. PDGFA Influences development of cephalic neural crest. (2000) Physiology and Biophysics Departmental Seminar, University of Miami School of Medicine, Miami, FL.

Belmont, HJ and Masters, BA. PDGFA influences patterning of cephalic neural crest cells. (2000) Southeast Society for Developmental Biology, Poster Presentation, Atlanta, GA.

Belmont, HJ and Masters, BA. Cephalic neural crest patterning is influenced by platelet-derived growth factor A-chain. (1999) Society for Developmental Biology, Poster Presentation, Charlottesville, VA.

Doc. 478304